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(54) Capillary zone electrophoresis cell system.

(57) A method and apparatus for increasing detector sensitivity in a Capillary Zone Electrophoresis detector is disclosed. A narrow bore capillary (10) includes an injection end (12) and an output end (30). Each end is placed in a reservoir (14,28) containing a buffer solution (15) and a sample of solute (16). The solute (16) comprises at least one unknown constituent component (17). An electric field is imposed across the buffer solution (5) and solute (16) in the capillary (10) by a power supply (22) coupled to leads (20,24) and electrodes (18,26). A source of ultraviolet light (38) illuminates a cell (32) within the capillary (10) and a sensor (42) measures the absorbance of radiation by the solute (16) to detect the constituent components(17).

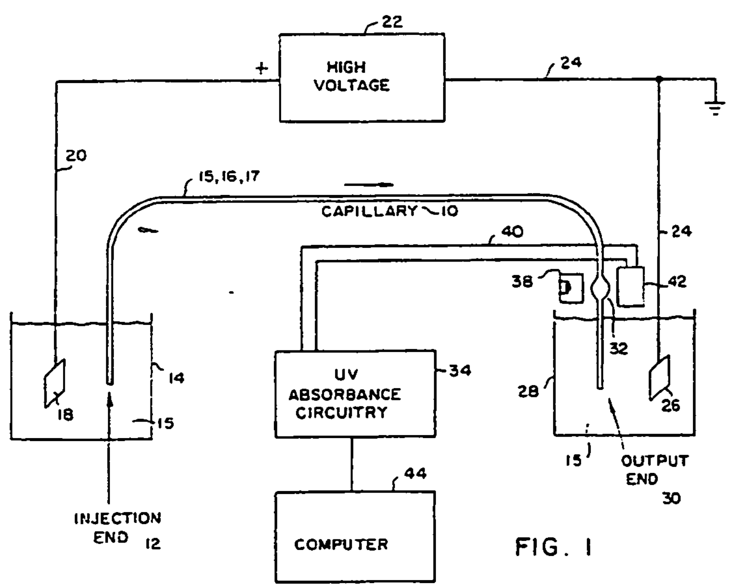

FIG. I

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CAPILLARY ZONE ELECTROPHORESIS CELL SYSTEM

The present invention is an apparatus and method for identifying unknown substances. This invention is particularly concerned with the recognition of large biological materials which are electrophoretically separated in a narrow bore capillary and which are then detected by measuring the amount of ultraviolet radiation absorbed by the unknown substances as they pass through a novel cell that resides within the capillary.

Recent advances in biotechnology have accelerated the demand of research laboratories, healthcare facilities, and pharmaceutical manufacturers for devices which are capable of accurately and rapidly identifying extremely small quantities of inorganic and organic substances. Previous techniques, which include gas and liquid chromatography, have been employed to assay samples whose molecular structure is relatively small. Although chromatography may be successfully employed to resolve metals, inorganic mixtures, and small organic ions; the very large and execeedingly complex molecules of amino acids, proteins, peptides, and DNA are more difficult to isolate and discover in a sample of unknown composition. Another serious flaw in chromatographic methods is the predicament that is encountered when the unknown sample is especially sparse, since chromatography utilises relatively large amounts of the material which requires analysis. Other shortcomings that are experienced when liquid chromatography is practiced include inconsistent patterns of solute movement through the system which creates uneven flows called "dead zones" and undesirable laminar mixing as opposed to bulk movement of the fluids.

Electrophoresis is another well-known procedure that enables the researcher or scientist to evaluate undetermined materials. An electric field is imposed across a length of tubing or capillary that contains a mixture of the unknown sample and a non-reactive liquid often called a buffer solution. The electric field causes the constituents of the unknown sample to migrate through the capillary due to the electrical attraction created by the field. Different components within the sample, however, are attracted at different rates due to their varying molecular drag and varying net electrical charges. Because dissimilar substances do not react to the drag and electrical attraction in the same way, they become increasingly separated into distinct zones or groups as they progress along the capillary. Each band of constituent material that makes up the original unseparated mixture of unknown material passes through the capillary by itself. At some point along this tubing, each band is examined and identified by a detector. One type of detector for electrophoretic separations measures the electrical conductivity of the bands in the capillary. An alternative detection scheme is a method called laser induced fluorescence. Although this technique is highly sensitive, it is costly and is limited to detecting compounds that fluoresce or which can be stimulated to do so. Another previous system probes the unknown material by shining light through the material and then gauging how much light is absorbed by the material. Unfortunately, the short and narrow path that the light travels across the capillary does not provide many opportunities for the undetermined sample to absorb photons. The capillaries must be kept very narrow so that all the material inside it moves easily without turbulence or eddy currents that might be caused by uneven radial heating across a larger tube. As a result of this size constraint, one major problem that plagues this approach is low detection sensitivity.

As the technology of genetic engineering continues to evolve, diagnostic and measurement techniques which are more accurate, reliable, and sensitive become increasingly more valuable. Doctors, clinicians, and laboratory technicians need more powerful tools to explore the intricacies of the genetic code, to improve the hardiness and usefulness of plant and animal life in an effort to feed the world's burgeoning population, and ultimately to devise cures for inherited disabilities and dreaded diseases. The problem of providing a highly sensitive and precise biological detection and analysis system that overcomes the limitations that impair previous devices and techniques has presented a major challenge to designers and innovators in the biochemical arts. The development of an effective, sensitive, affordable, and unerring system for sensing the components of an unspecified biological sample would constitute a major technological advance in the biochemical and biotechnology industries. The enhanced performance that could be achieved using such an innovative device would satisfy a long felt need within the business and would enable manufacturers of drugs, medicines, and biological products to save substantial expenditures of time and money.

The Capillary Zone Electrophoresis Cell System disclosed and claimed in this patent application provides accurate detection of unknown biological samples at sensitivity levels and cost constraints which are unattainable using conventional analysis methods. The key to the enhanced performance that is achieved using the present invention is a novel cell which is integrally formed within the

detection circuit of the narrow bore capillary. The Capillary Zone Electrophoresis Cell System is capable of sensing injected substances at the remarkable sensitivity threshold of one part per million concentration. This achievement has been accomplished with an system that is economical, easy to use, and highly reliable.

A tiny capillary with an inside diameter about as thin as a hair conducts a carrier fluid from an injection end to an output end. Both ends of the capillary are placed in reservoirs which also contain the carrier fluid, which is usually called a buffer solution. At the start of the separation, micro-grams of an unknown substance are inserted into the injection end of the capillary. An electric field is then imposed across the capillary. Due to the electrical field, charged component parts of the unknown sample move from one end of the capillary to the other end. During the last stages of their migration, the unspecified constituents of the sample enter an egg-shaped cell. While in the cell, ultraviolet light is directed toward and through the cell. Part of the light is absorbed by the biological sample. The level of absorbtion is measured by a detector, and, based upon comprehensive and widely-available knowledge which indicates which types of substances absorb various amounts of light, an analysis of the sample is obtained.

The Applicant's Capillary Zone Electrophoresis Cell System is a diagnostic and measurement device that offers an unprecedented sensitivity in detecting the constituent factors of microscopic samples. The apparatus claimed in this patent application provides an effective, efficient, and powerful tool that will enable engineers and scientists in the medical instrumentation industry to construct analysis equipment that will revolutionize the critically important field of biotechnology.

An appreciation of other aims and objectives of the present invention and a more complete and comprehensive understanding of this invention may be achieved by studying the following description of a preferred embodiment and by referring to the accompanying drawings.

Figure 1 is a schematic diagram that illustrates a Capillary Zone Electrophoresis Cell System.

Figure 2 is a side view of the cell showing the electric field and isopotential lines.

Figure 3 is a side view of the cell that also reveals bands of solute borne by the buffer solution passing through a beam of ultraviolet radiation.

Figure 4 is a schematic comparison of photon flux and path length through three different size capillary cross-sections.

Figure 5 is a comparison of normalized detected bands showing the reduction in noise achieved by the using successively larger cell diameter sizes shown in cross-section in Figure 4.

Figure 6 is a graph which plots detector response against the concentration of a detected solute and which illustrates the desirable linear characteristic of the present invention.

Fife 7 ia a graph which reveals the sensitivity of the Capillary Zone Electrophoresis Cell System. The sharp drop in the curve from left to right shows the abrupt change in absorbance of a protein equivalent present in the cell at a concentration level of only one part per million.

Figure 8 is a schematic side view of a glass lathe that is employed to fabricate the novel CZE cell.

Figure 1 is a schematic illustration of a Capillary Zone Electrophoresis (CZE) Cell System which includes a narrow bore capillary 10 having an injection end 12 located in an injection reservoir 14. The capillary 10 is fabricated from fused silica and measures about 50 microns across its inside diameter, although different-sized micro-bore capillaries are envisioned by the inventor. Injection reservoir 14 supplies the capillary 10 with an electrolytic aqueous medium of constant isocratic solution, such as a simple salt or buffer solution 15. A sample 16 having unknown constituent parts 17 is inserted into the injection end 12 by momentarily dipping the end 12 into a sample vial (not shown) and by drawing a small amount of sample 16 into the capillary 10 by the application of voltage or pressure. A high voltage power supply 22 is connected to a positive electrode 18 which is situated in injection reservoir 14. A ground lead 24 connects the power supply 22 to a negative electrode 26, which is similarly located in an output reservoir 28. An output end 30 of capillary 10 is placed in output reservoir 28.

A novel cell 32 is formed within the capillary 10 and is illuminated by a source of ultraviolet (UV) light 38. Throughout the specification and claims, the terms "containment means", "dilated zone", and "cellular containment means" refer to the cell as pictured in the drawings and as described by the specification. A detector 42 receives light from source 38 after it passes through the cell 32 and is absorbed by components 17 of any solutes 16 that are carried in the buffer solution 15. The detector 42 is connected to ultraviolet absorbance circuitry 34 by leads 40. Measurements may be interpreted and displayed by a computer 44 or by a strip-chart recorder (not shown) which receives signals from the absorbance circuitry 34.

Figure 2 reveals one embodiment of the novel cell 32 in great detail. The smallest diameter of the cell 32 is represented by the distance across the pairs of arrows labeled "46". In the best mode of the invention, this dimension is fifty microns and matches the inside diameter of the narrow bore

capillary 10 and is a part of the capillary 10. The greatest diameter of cell 32 is slightly larger than capillary 10 and is indicated by the pair of arrows labeled "48". In this embodiment, the maximum diameter 48 of the cell 32 is one hundred and fifty microns. A curved portion of the cell 32 is delineated by reference numeral 50. The end points of the curved portion 50 of the cell 32 are marked by reference numerals 52 and 54. A distance 56 shown in the upper left portion of Figure 2 represents the longitudinal projection of the curved portion 50 of cell 32. The total length of the cell 32 is marked by reference numerals 57, which appear at the bottom of Figure 2. Horizontal lines 58 illustrate electric field lines generated by the influence of power supply 22 and electrodes 18 and 26. Vertical lines 60 reveal lines of equal potential. In this illustration, the volume of the cell 32 is equivalent to the volume of 1.6mm of capillary length. This volume is measured in nanoliters. This remarkably tiny volume utilized by the Capillary Zone Electrophoresis Cell constitutes an improvement of a full three orders of magnitude of miniaturization over cells fabricated for liquid chromatography detection.

Figure 3 depicts yet another embodiment of the CZE cell 32 which has an elongated central portion 61 that extends in either direction to a narrow portion 74. The outside diameter of capillary 10 is indicated by the reference numeral "62". A beam of ultraviolet radiation 64 is directed toward the center of the cell 32 so that the of the radiation 64 is perpendicular to the longitudinal axis of the capillary 10. The wavelength of the radiation employed is usually in the range of 200 to 400 nanometers (nm). The band at the right side of the center of cell 32 represents a first distinct group of solutes or analytes 16 migrating from left to right, typically comprising a tenth of a unit of volume called a "peak". These bands of solute, labeled 66 and 68, are often referred to as "fractions of peaks" because they collectively correspond to a graphic peak or spikes of an electropherogram which displays increased levels of UV light absorbtion along an abscissa scaled in detector voltage, and across an ordinate marked in units of time. As each constituent part 17 of an unknown sample 16 passes through the cell 32, the light absorbed by the constituent parts 17 increases, and reduces the amount of radiation that falls on the sensor 42. The resulting graph is a series of Gaussian distributions which identify the materials in the capillary 10. In this illustration, each band 66 and 68 shown in Figure 3 represents one-tenth of a the width of a typical narrow peak

Figures 4(a), (b), and (c) exhibit the cross-sections of three combinations of outer capillaries and enclosed cells having increasingly larger diameters available for conduction of the buffer solution 15 and the solute 16. Capillary outside diameters 76, 78, and 80 correspond to a 50 micron cell 82, a 150 micron cell 84, and a 200 micron cell 86, respectively. The cross hatching shown within each cell 82, 84, and 86 represents the flux density of photons striking the cells which are available for absorbtion. The increased optical path length provided by the larger bores contributes directly to an increase in sensitivity and a concomitant decrease in noise levels. The sensitivity increases by the multiple of enhanced photon path length and by the square root of the path's cross-sectional area, due to the larger flux of radiation. If cell 82 in Figure 4(a) is taken as a reference, cell 84 provides an increase in sensitivity of $3\sqrt{3}$ or nearly five times an improvement in sensitivity. The 200 micron cell 86 develops a sensitivity capability over eight times that of the reference cell 82, based on the product of the increased path length, 4, and the larger radiation density $\sqrt{4}$. The improvement is actually even greater than this value, since the present invention avoids the problem of stray light-- flux that spills around the sides of the cell-- which disproportionately penalizes smaller previous cells.

The three curves 88, 90, and 92 depicted in Figure 5 correspond to the results of employing cells 82, 84, and 86 shown in Figure 4. The three curves are normalized to show the progressive reduction in signal noise. Curve 92, a consequence of using the 200 micron cell 86, reflects a substantially depressed noise level compared to curve 88, which reports the results of using the reference cell 82 which is only 50 microns across. One penalty incurred by increasing the size of the cell is the broadening of the peaks. Figure 5 shows that the width of peak 92 is slightly larger than that of peak 88. The degree of broadening experienced in this sample is appropriately ten percent. Since the resolution of the CZE detector is measured by the separation between peaks, broader peaks degrade the performance of the system. The ten percent degradation seen here, however, is relatively insignificant compared to the ten-fold gains in sensitivity achieved by employing the novel CZE cell which provides for increased optical path length and flux.

Figure 6 exhibits the strong and desirable linear characteristic of the present invention. Curves 94 and 96 are graphs of detector response in millivolts (mV) for a sample absorber, DMSO. Curve 94 was generated using a log amplifier in the measurement equipment. The use of a log amplifier introduces noise to the system, but the resulting plot shows greater linearity at strong concentrations. Curve 96 was generated using a linear amplifier. This mode of measurement offers lower noise and higher sensitivity. A log operation can be

performed after the signals are amplified above the circuit noise level to combine the sensitivity of curve 96 with the linearity of curve 94. The extended linearity of both curves 94 and 96 is far greater than that attainable with previous methods that utilize detection on conventional capillaries without the novel cell 32 claimed in this application, because the novel cell 32 helps to eliminate stray light that would otherwise skirt the sides of a much smaller target and that would not pass through the solute 16.

Figure 7 reveals the striking results of placing less than one part of average protein equivalent in a million parts of buffer solution and passing the mixture through the detector. The left portion of the curve 98 is a detector output showing a first level of absorbance of pure buffer solution. When the one part per million of protein equivalent passes into the CZE cell, the absorbance increases dramatically, as indicated by the reduced detector output shown by the right portion of the curve, labeled 100. This one drawing vividly expresses the power of the present invention to sense complex biological substances in minute concentrations.

Figure 8 is a schematic side view of a cell lathe 102 that is employed to fabricate the novel CZE cell 32. The lathe 102 includes a base 104 that anchors motor 106 which is connected by a drive shaft 107, supported by bearing 110, to drive gears 108. Drive gears 108, in turn, are coupled to driven gears 112 and 114, which are mounted on rotating hollow shafts 113 supported by bearings 116. Chucks 118 are mounted on hollow shafts 113 and provide support for capillary 120 at two points. Capillary 120 is bolstered by support 122 and is held by a pair of chucks 118. The operator of the lathe 102 views a central section of capillary 126 that is heated by a small flame through microscope 124. Micro-blowing methods that scaled-down versions of techniques which are well-known to persons ordinarily skilled in the glass fabrication art were employed to manufacture the CZE cells 32. The capillary 120 is first sealed off at one end, and then a syringe 128 is used at the other end to pressurize the volume within it to ten to twenty percent over-pressure. A high quality cell 32 may be formed by rotating the capillary 10 in a 1/16" gas flame for three or four seconds.

Operation of the CZE System

The motivating force which drives the separation of analytes in the present invention is an electric field which is imposed across the capillary. A potential difference of thirty to fifty kilovolts causes substances borne by the carrier to move from one end of the tube to another, but the movement itself has two components. The more apparent and more readily understood of the two is electrophoretic migration. Electrophoresis refers to the process of charged molecules moving toward an oppositely charged electrode due to the simple electrical attraction of dissimilar charges. The second reason for movement of materials through the CZE system is a phenomenon called electro-osmotic flow. Whenever a liquid is placed in a glass tube, molecules in the liquid that have a negative charge tend to stick to the walls of the tube. Positive charges do not behave this way. This preferential sticking or adsorbtion of negatively charged molecules attracts a thin layer of positively charged molecules all along the inner walls of the capillary. The electric field pulls these positive molecules or ions toward the negative electrode at the output end of the capillary. The positive ions drag other molecules along with them, even those having a neutral or negative charge. The result is a flow of all kinds of differently charged solute moving toward the output end of the capillary. Electro-osmotic flow provides the pumping force which moves molecules of all charges toward one end of the system.

The strength of the electro-osmotic force that generates the bulk flow in the capillary is directly proportional to the applied electric field. Within the CZE cell, the field is lower than the strength of the field in the capillary due to the larger cross-section of conductive fluid within the cell. Since the field is inversely proportional to the fluid area, the field is only one sixteenth as strong within a cell that has a diameter that is four times greater than that of the capillary. In this cell, the osmotic force at the capillary wall within the cell is also sixteen times weaker than the osmotic force at the walls in the rest of the capillary. If the flow were constant, this force would be too small and would result in a laminar flow in which solute at the edge of the cell would lag behind solute in the center of the cell. This uneven transport would cause undesirable spreading of the peaks and would degrade the performance of the CZE system. As a direct consequence of the design of the present invention, this problem does not occur. As this osmotic bulk-flow force drops in the cell, the flow velocity in the cell also drops in proportion to the cross-sectional area of the cell. When the bulk flow slows down in the cell, the forces that propel it fall concomitantly. The extremely beneficial result is a balance of forces on the bands of analyte and an absence of forces that would tend to distort the bands and broaden the peaks.

Design Considerations

- Turbulence

One of the primary concerns taken into account when the cell 32 is designed and fabricated is reduction of turbulence. An important feature of the invention is the gradual curvature of the cell. Sharp edges, fittings, gaskets, or joints in the capillary circuit, which are common in liquid chromatography systems, would also denigrate the performance of the detector and are avoided by providing gently sloping curves and transitions. The smooth flow that results from the careful observance of these design constraints avoids turbulence.

- Capillary size

The two characteristics that contribute to the quality of a separation of materials in the capillary are selectivity, the ability to definitively separate similar solutes, and sensitivity, the degree to which small amounts of solute can be detected. A CZE system can be made more sensitive by increasing the diameter of the capillary. Larger bore capillaries are more sensitive because their greater diameter supplies a longer path length for light to pass through them. As a consequence, more opportunities are provided for the solute to absorb ultraviolet radiation. The same systems can be made more selective by reducing the diameter of the capillary. Small bore capillaries below 50 microns in diameter do not experience the undesirable radial temperature gradients that are created by the excessive heat which is generated in larger systems. These radial temperature gradients cause the analyte in the center of the capillary to migrate faster than the analyte at the edge, which broadens the peaks and degrades resolution. The unwanted thermal gradients adversely affect molecules of solute as they are being separated by reducing the sharpness of the boundaries of the bands of solute that form in the capillary. When the definition of these bands is compromised, the solute "peaks" become broadened, and the selectivity of the separation is degraded. The novel method and apparatus claimed and disclosed in this patent application resolve the conflicting design considerations concerning selectivity and sensitivity by providing superior performance without having to trade one measure of quality for another.

- Advantages over Liquid Chromatography-Type Cells

The present invention offers several significant improvements over conventional liquid chromatographic cell fabrication techniques. Liquid chromatography (LC) is limited by a cell size that is one thousand times greater than CZE and by a cell geometry that produces cells with sharp corners known as "unswept volumes". These pockets cause solutes to hang in the cell, to broaden the detected peaks, and to degrade selectivity. Another disadvantage of LC cells is that the flow through them is laminar-- the buffer at the center of the cell moves the fastest, while the buffer at the edge sticks to the edge of the cell. In theory, the flow develops a parabolic velocity profile across the tube in which it flows. This nonlinear profile impairs the performance of the cell because the shape of each band of solute is distorted by this uneven flow. The LC cell must, therefore, be nearly an order of magnitude smaller than the smallest LC peak volumes, so that the peaks are not broadened and selectivity is not sacrificed.

The Capillary Zone Electrophoresis Cell System provides an accurate, powerful, and reliable tool for diagnosis and measurement across a broad spectrum of medical and manufacturing applications. This invention constitutes a major step forward in the continually evolving fields of biotechnology and chemistry.

Claims

1. An analytical apparatus comprising: a tubular vessel (10) capable of receiving a transport medium (15) and a substance (16) comprising at least one constituent component (17); an electric field generator (18,20,22,24,26) which imposes an electric field across said tubular vessel (10); and a radiation source (38) and a radiation sensor (34,40,42) that together measure the effect on radiation passed through a portion of said tubular vessel.

2. An apparatus according to claim 1, wherein the tubular vessel (10) has a dilated zone (32), forming a cell, located between an injection end (12) and an output end (30) of said tubular vessel (10); and the effect on radiation passed through said cell is measured by said radiation source (38) and sensor (34,40,42).

3. An apparatus according to claims 1 or 2, wherein said tubular vessel comprises a narrow bore capillary (10) filled with said transport medium comprising a buffer solution incorporating a substance (16) including at least one constituent component (17), the radiation source and sensor are incorporated in an ultra-violet absorbance detector (34), and the apparatus further comprises an injection reservoir (14) disposed to receive said injection end (12) of said capillary (10) and an output reservoir (28) disposed to receive said output end (30)

of said capillary (10); both of said injection and said output reservoirs (14,28) being capable of holding said buffer solution (15) and said substance (16); and a power supply (22) coupled to a first electrode (18) and to a second electrode (26).

4. An apparatus according to claims 2 or 3 in which said cell (32) has a cell length (57) that is approximately equal to the inside diameter (48) of said cell (32).

5. An apparatus according to any of claims 2 to 4 in which said cell (32) has a curved portion (50) having a longitudinal projection (56) of said curved portion (50) that is within an order of magnitude of an outside diameter (48) of said cell (32).

6. An apparatus according to any preceding claim, wherein said capillary (10) is ultra-violet transparent, said cell (32) is integrally formed with said capillary, said electric field generator (18,20,22,24,26) acts as an electrophoretic means for moving said substance (16) through the capillary, said radiation source (38) illuminates a central part of said cell, and the apparatus further comprises amplification means (34,44) responsive to said radiation sensor (34,40,42) for displaying an electronic output that indicates the concentration of said substance in the cell.

7. An apparatus as claimed in any preceding claim, in which said radiation sensor (42) is a photodiode.

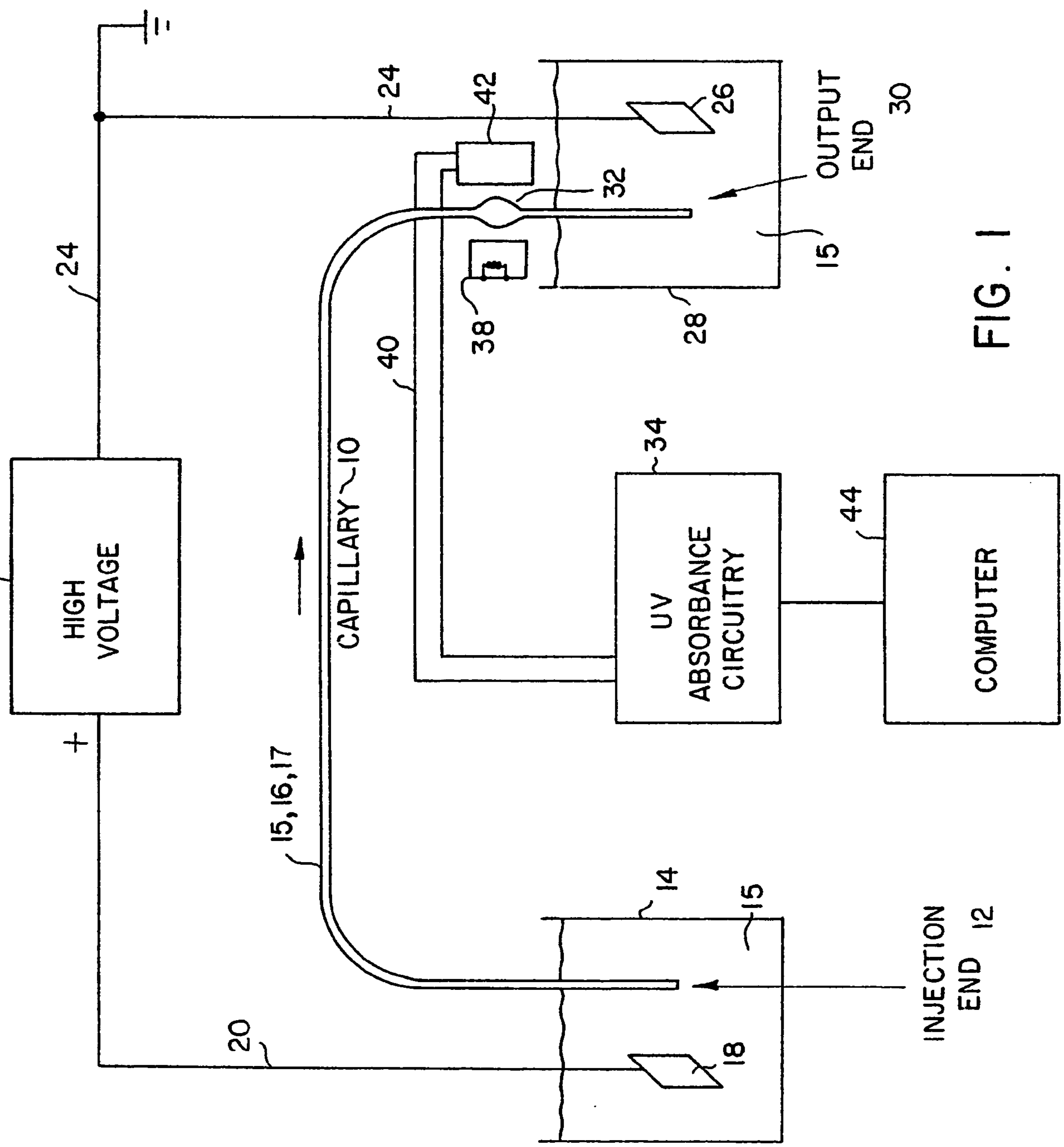
8. An apparatus as claimed in any of claims 1 to 6, radiation sensor (42) is a photomultiplier tube.

9. An apparatus as claimed in any of claims 1 to 6, in which said radiation sensor (42) senses an absorbtion of ultra-violet light by said substance (16).

10. An apparatus as claimed in any of claims 1 to 6, in which said radiation sensor (42) senses a fluorescence light emitted by said substance (16).

11. A method of identifying an unknown sample comprising: providing a tubular vessel (10) capable of holding a transport medium (15) and a substance (16) having at least one constituent component (17); imposing an electric field across said tubular vessel (10) to move said transport medium (15) and said substance (16) through said vessel (10); directing a beam of radiation toward said substance (16) while it passes through a cell (32) within said vessel (10); and measuring a level of radiation absorbed by said substance (16) to identify said constituent component (17).

12. A method of fabricating a cell (32) comprising a part of an apparatus according to any preceding claim, the method comprising the steps of heating a small portion of said capillary (10); and pressurizing said capillary to dilate said capillary to form said cell (32).



HIGH VOLTAGE
22
+
24
24
CAPILLARY 10
15,16,17
20
14
15
18
INJECTION END 12
42
32
26
OUTPUT END 30
15
38
28
40
UV ABSORBANCE CIRCUITRY
34
44
COMPUTER

FIG. 1

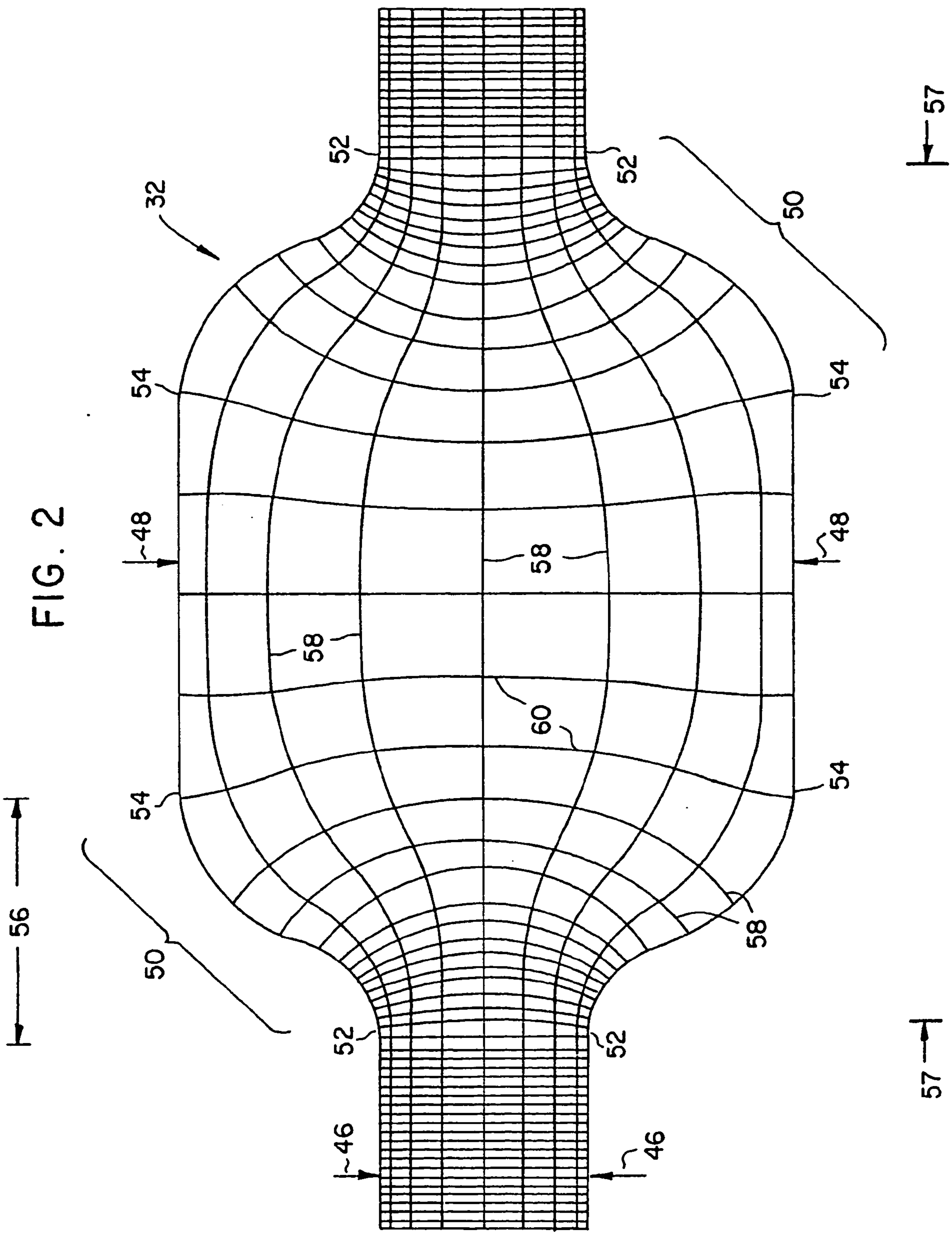


FIG. 2

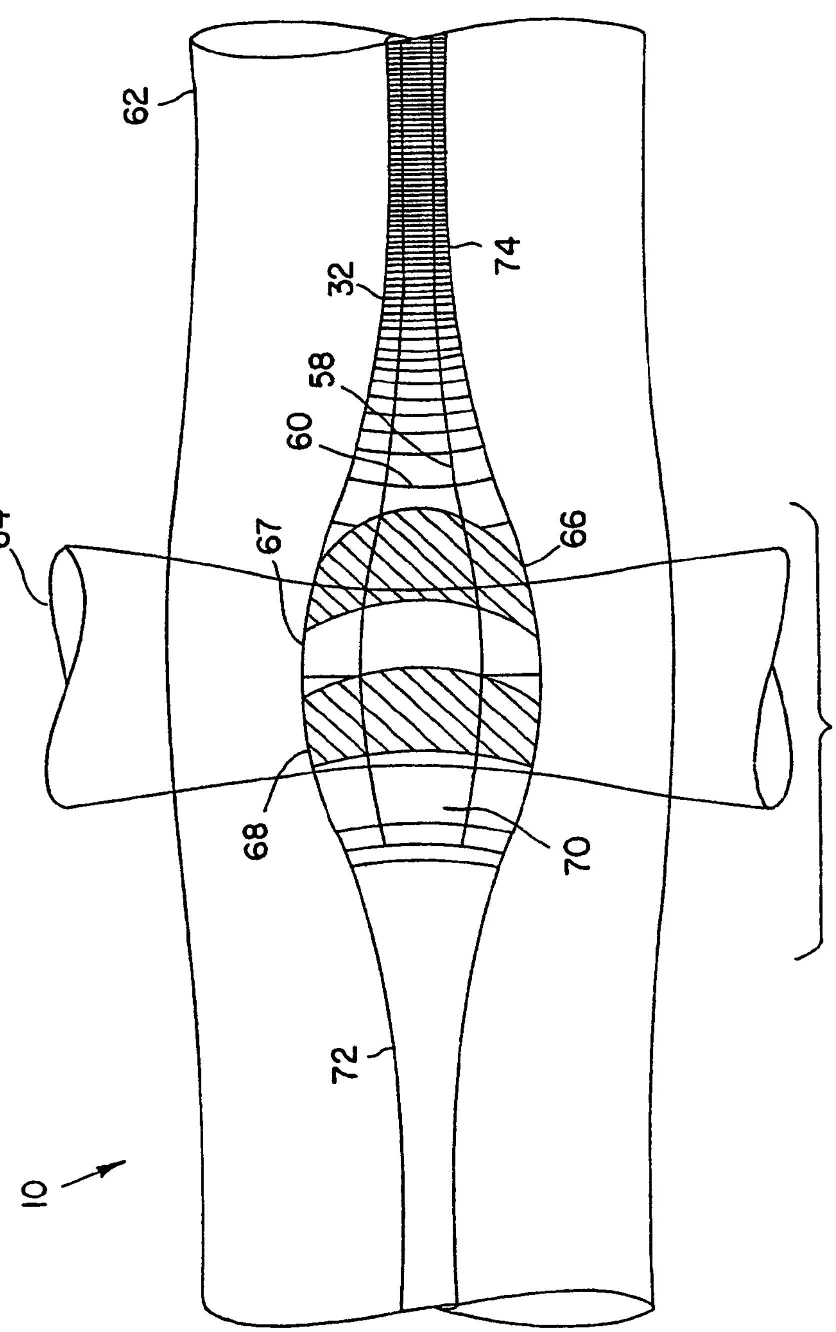


FIG. 3

FIG. 4A

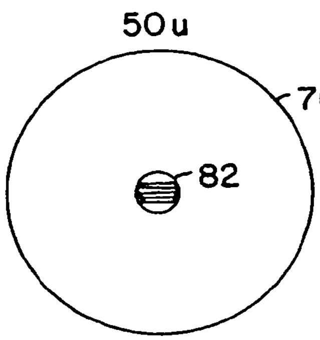


FIG. 4B

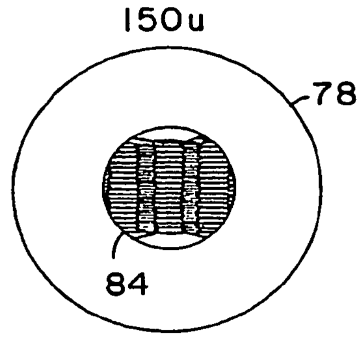


FIG. 4C

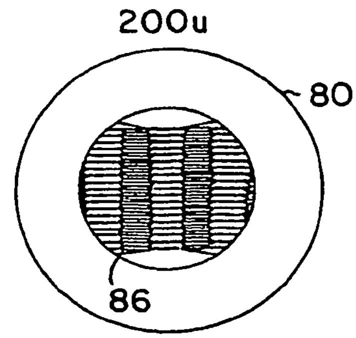
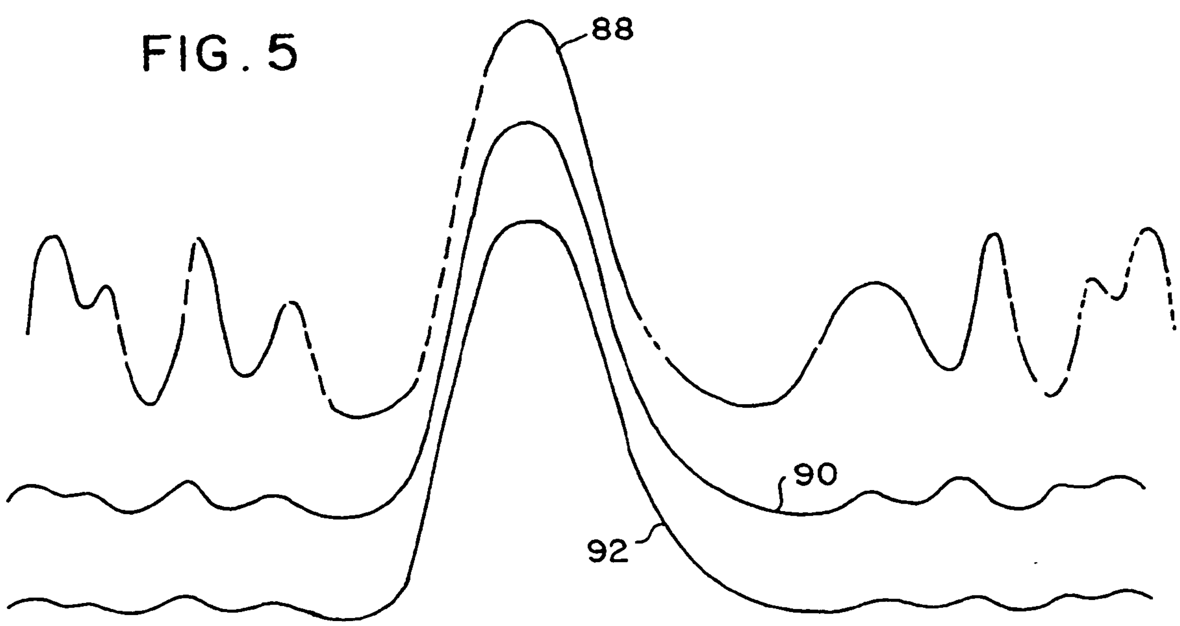


FIG. 5



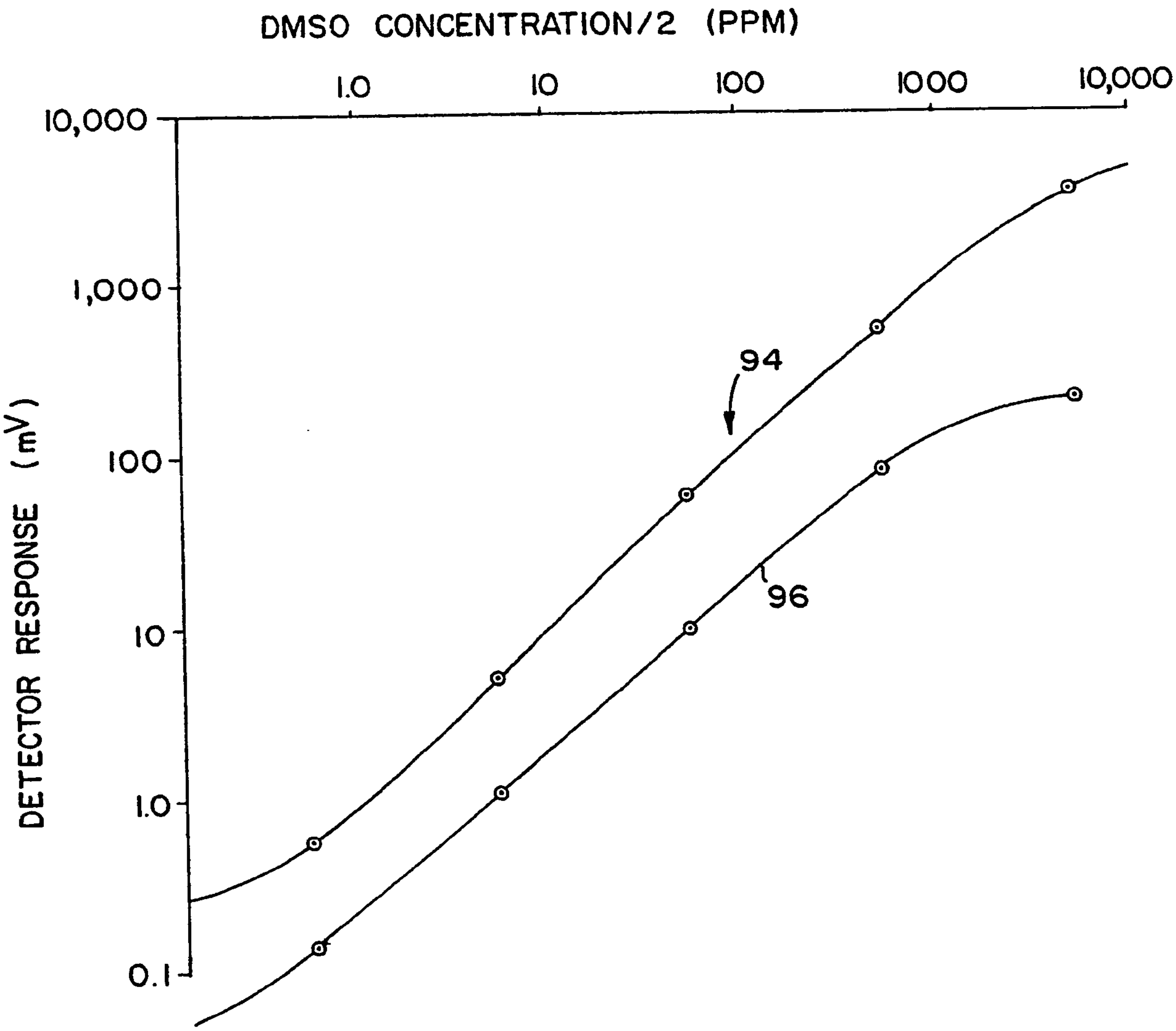

DMSO CONCENTRATION/2 (PPM)
1.0
10
100
1000
10,000
10,000
1,000
100
10
1.0
0.1
DETECTOR RESPONSE (mV)
94
96

FIG. 6

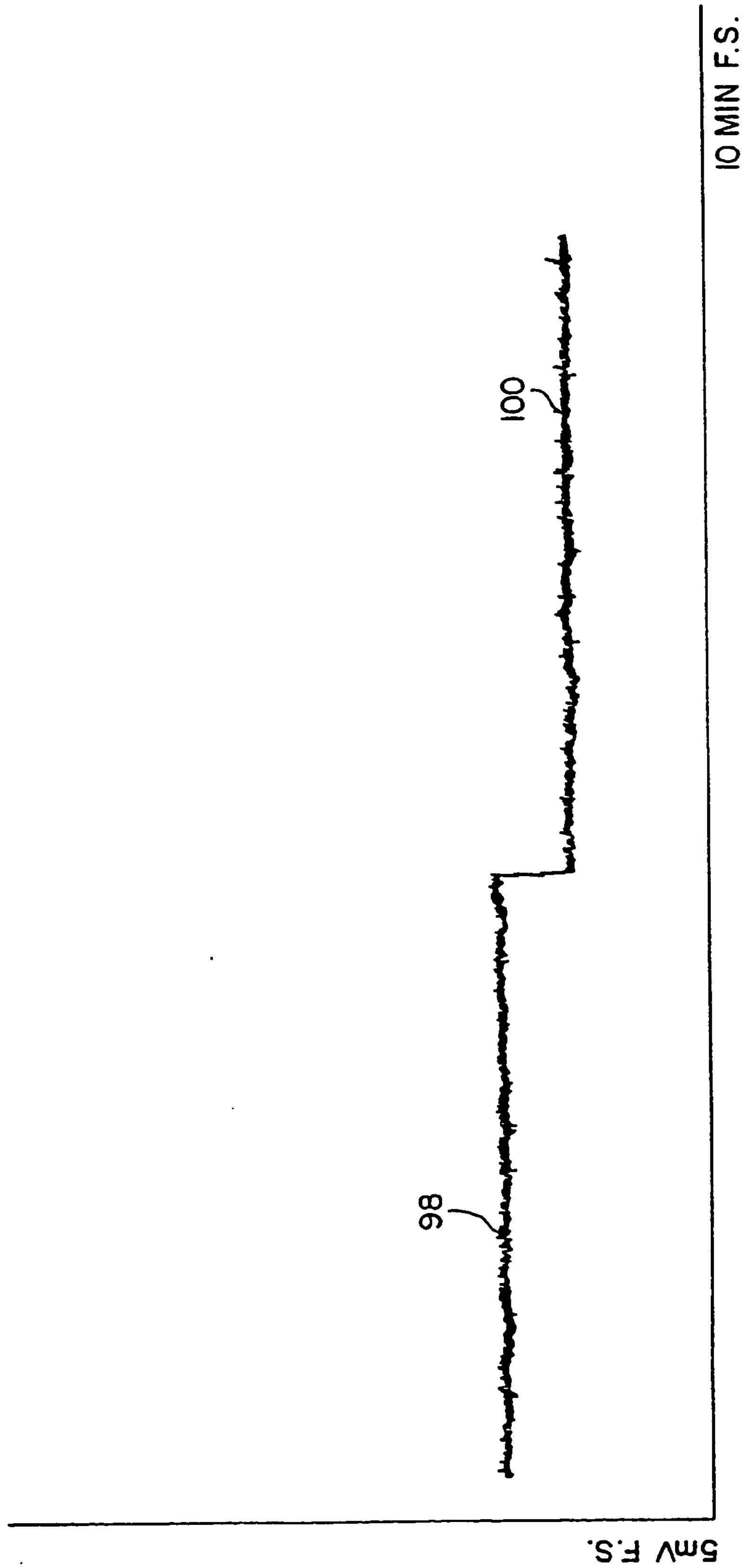


FIG. 7

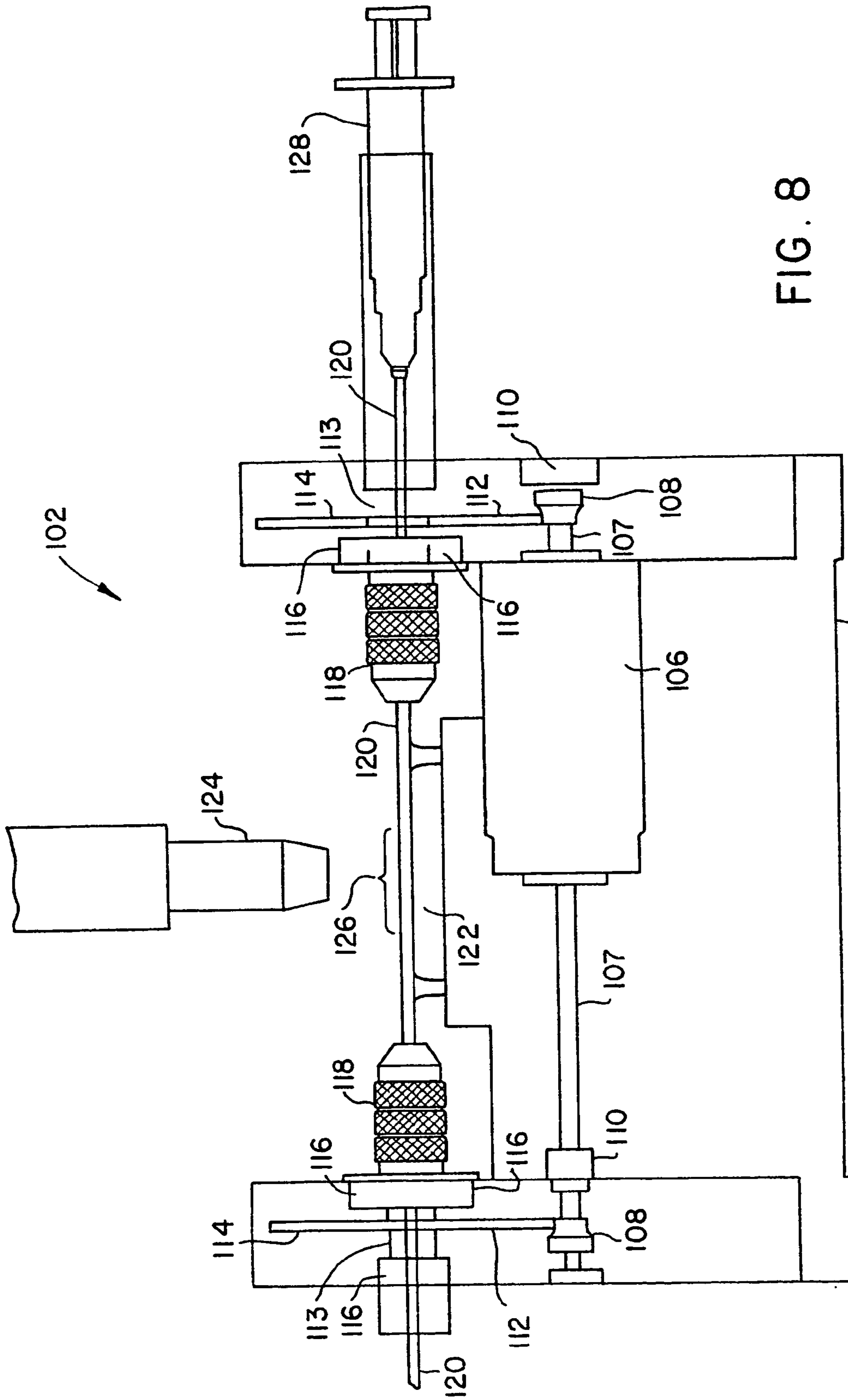


FIG. 8

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 30 2093

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
A	APPLIED SPECTROSCOPY, vol. 43, no. 2, February 1989, pages 196-201, Society for Applied Spectroscopy, Frederick, MD, US; M. YU et al.: "Attomole amino acid analysis: Capillary-zone electrophoresis with laser-based thermo-optical detection" * Pages 196-201 *	1	G 01 N 27/447
A	EP-A-0 216 600 (R. ZARE) * Figures 3,4 *	1	
A	FR-A-2 558 262 (E. LEROY) * Figure 3 *	1	
			TECHNICAL FIELDS SEARCHED (Int. Cl.5)
			G 01 N

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	14-06-1990	DUCHATELLIER M.A.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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